

## OncoPet Serum RECAF Test Results Using Canine Serum Specimens.

### OncoPet Diagnostics Inc.

Cancer and fetal cells from different species - but not their normal adult counterparts – express a receptor for AFP (RECAF). The binding site for AFP on this receptor is the glycan (sugar) portion attached to a number of different glycoproteins, and it is rather conserved over the zoological scale since AFP from one species binds to RECAF of other species, albeit with lower affinity. Hence, the monoclonal antibody, 1.4G11, which is directed against the AFP binding site on RECAF, recognizes not only human RECAF, but also piglet, rat, mouse and dog RECAF (and possibly cat RECAF).

BioCurex, our parent company, has previously developed Radioimmunoassay (RIA) and Chemiluminescence (CIA) serum assays for measuring circulating RECAF in humans. BioCurex has accumulated a large amount of data demonstrating that RECAF is elevated in cancer patients as compared to normal, healthy donors. Also, some benign lesions, such as those of prostate, breast and stomach are in general – but not always - negative.

Thus, using the same antibody, we developed a CIA RECAF test for the analysis of canine serum specimens. The principle of the assay involves the competition of labeled RECAF with RECAF in the serum sample for binding to the anti-RECAF monoclonal antibody on the solid phase.

Some definitions we shall use throughout this document:

*Sensitivity* is the percentage of cancer samples that tested positive over the total number of cancer samples. For example, if the test detected 780 cases out of 1000 cancer samples, then the sensitivity is 78%. Correctly diagnosed cancer samples are called True positives (TP). Cancers that were missed by the test are False Negatives (FN). Thus, sensitivity can also be expressed as **Sensitivity = TP / (TP+FN)**.

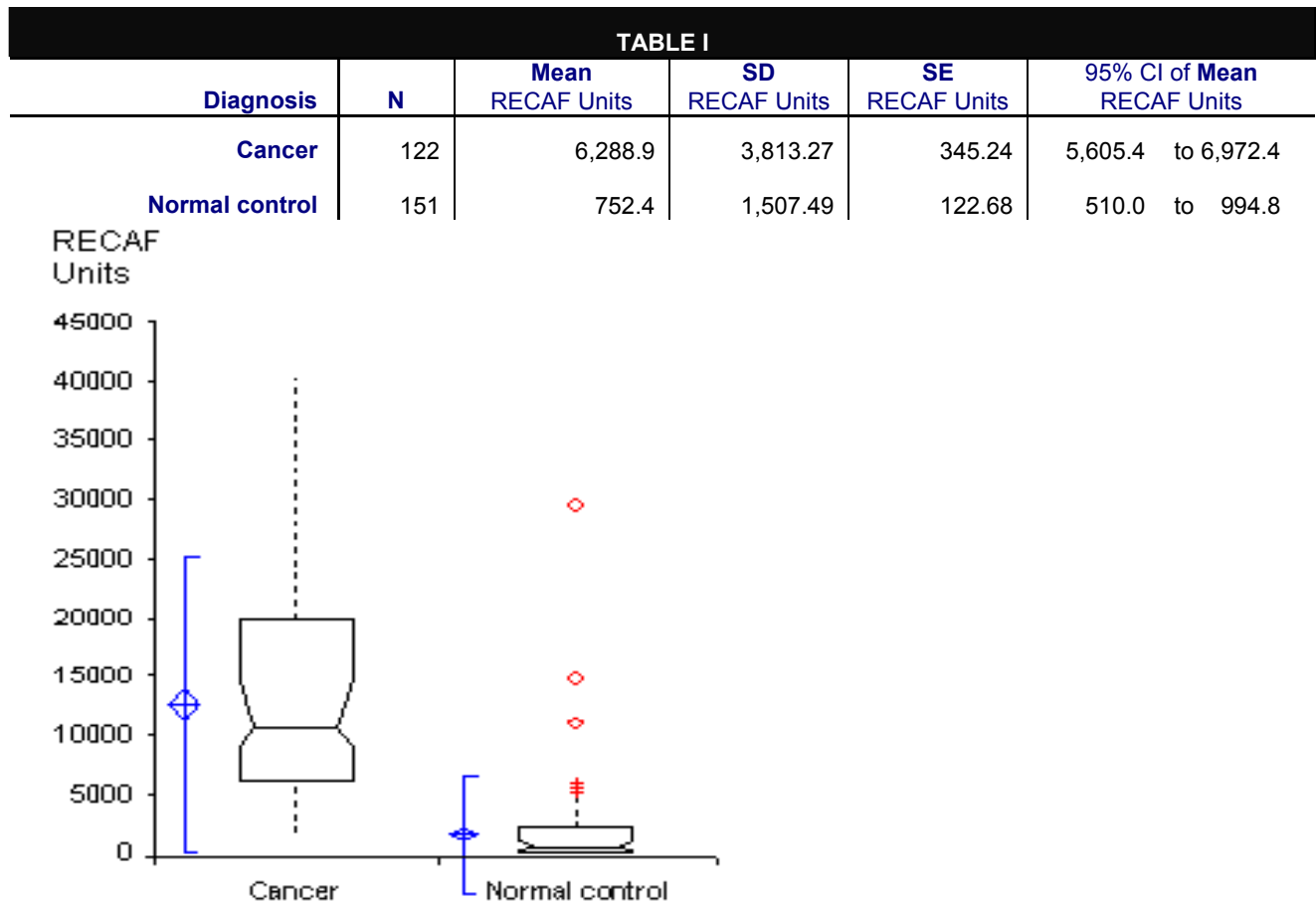
*Specificity* is the proportion of normal samples that test negative and it can be expressed as **Specificity = TN / (TN+FP)**. Where TN = True Negative (normal subjects detected as negative) and FP = False Positives (the healthy subjects incorrectly tested as positive)

ROC (Receiver Operating Characteristic) curve:

The ROC curve plots Sensitivity vs. Specificity (actually 1 - Specificity to get the curve to start on the left side of the graph). It represents the ability of the test to discriminate cancer from normal samples. A test with no discrimination whatsoever (e.g. the +/- result is at random, like tossing a coin) results in a diagonal line across the axes. As the assay discriminates more and more, the curve “bulges” up. A test with perfect discrimination (100% sensitivity and 100% specificity) results in a graph in the shape of a straight angle. For a detailed explanation of ROC curves, see Appendix A.

**Example of results obtained on dogs:**

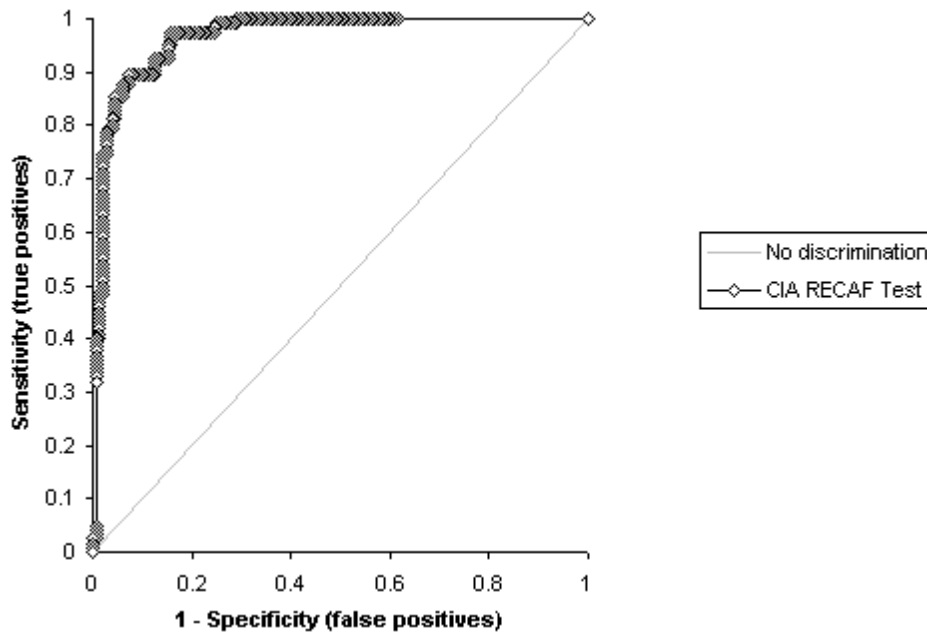
1. Approximately 300 dog sera were tested. These specimens were supplied by seven different clinics or other veterinary organizations and they were taken from normal animals (n = 151) or dogs with confirmed cancer (n = 122).
2. We also received a few benign tumor samples, most of which were negative, but the numbers were too small to draw any statistical conclusion.
3. The Sensitivity of this test for Cancer vs. Normal Specimens, from all 7 locations, was determined to be 85% at 95% Specificity. See Figure 1 & 2.
4. Regarding the serum specimens from the largest supplier in the study (83 Cancer and 27 Normal) the Sensitivity was 99% at 100% Specificity. (Fig 3.)



**Figure 1. Cancer vs. Normal Animal Serum Specimens comparative descriptive for all 7 Donor Organizations.**

**Figure 2. Receiver Operator Characteristic (ROC) Curves for Cancer vs. Normal Animal Serum Specimens for all 7 Donor Organizations.**

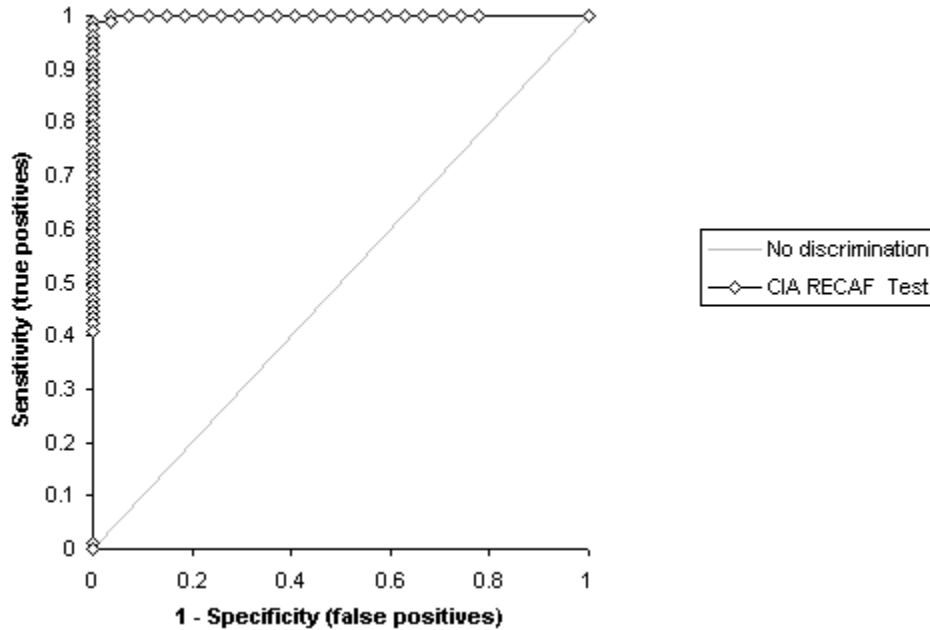
CIA RECAF Test by Diagnosis					
n	273				
<b>Diagnosis</b>	<b>N</b>				
Normal control	151				
Cancer	122				
<b>Curve</b>	<b>Area</b>	<b>SE</b>	<b>p</b>	<b>95% CI of Area</b>	<b>Diagnosis = Cancer</b>
CIA RECAF Test	0.966	0.0101	<0.0001	0.947 to 0.986	have higher values



**Figure 3. Receiver Operator Characteristic (ROC) Curves for Cancer vs. Normal Animal Serum Specimens of one of the suppliers only.**

CIA RECAF Test by Diagnosis	
n	110
<b>Diagnosis</b>	<b>N</b>

Normal control	27					
Cancer	83					
Curve	Area	SE	p	95% CI of Area	Diagnosis = Cancer	
CIA RECAF Test	1.000	0.0008	<0.0001	0.998 to 1.000	have higher values	



While these results strongly suggest that the test performance is consistent with its use for dog cancer screening, diagnosis and follow up, the following limitations should be considered when interpreting the results:

**DISCLAIMERS:**

**Please read this carefully:**

1. The OncoPet RECAF™ test should **NEVER** be used to test any species, human or animal, other than dogs.
2. The OncoPet RECAF™ test has **NOT** been approved by the U.S. Food and Drug Administration, the U.S. Department of Agriculture, or any other regulatory organization in any country for use in the diagnosis of cancer in any species and there is no assurance when or if such approval will be forthcoming.
3. The information provided on this web site is for general information purposes only and is not intended as medical or veterinarian advice. Medical or veterinary advice regarding companion animal cancer and its appropriate treatment should only be obtained from a qualified licensed veterinarian.

4. There is insufficient data at this time to assess the results of using OncoPet RECAF™ in dogs with benign neoplasia. The limited information available suggests that 10% -15% of benign lesions could test positive, which is consistent with the values obtained in human samples. At this time it is unknown if RECAF™ positive benign tumors are in the process of becoming malignant.
5. The calculation of the positive/negative cutoff value and the sensitivity and specificity of the test are estimated from a relatively small number of samples and might not reflect the values obtained from a larger number of samples and might require adjustment as the number of samples increases. The efficacy of screening larger populations has yet to be determined. An elevated RECAF™ level can occur in some non-malignant neoplasms as well as in advanced and terminal cancer, where the benefits of using the test are nil. Acute infections can sometimes trigger an elevation of RECAF™ levels and therefore these should be eliminated by other diagnostic procedures.
6. The OncoPet RECAF™ test is **NOT**, by itself, conclusive of the presence or absence of cancer and its results can only be assessed as an aid in the detection or monitoring of cancer in relation to the history, medical signs, symptoms and the overall condition of the animal.
7. As with other cancer markers, the chance of having cancer varies with the amount of circulating RECAF™. Thus, an animal with high RECAF™ values is more likely to have cancer than an animal with low RECAF™ values. The error in estimating whether a patient is positive or negative for cancer is highest around the cutoff value used as stated in the report to the veterinarian.
8. None of OncoPet Diagnostics, Inc., BioCurex, Inc. or any of their respective, directors, officers and employees, will be liable for the content of the reported results or for the consequences of any actions taken on the basis of the information provided. Any reliance you place on such information is strictly at your own risk.
9. OncoPet Diagnostics Inc. does not accept samples sent by any other person than a qualified veterinarian. The company reserves the right not to test samples sent for testing and in that case, it is under no obligation to return the sample or other collection material.
10. **Guarantee:** OncoPet Diagnostics, Inc. will provide an OncoPet RECAF™ test for free for each false positive or false negative as established upon receiving a contradictory report from an independent pathologist.
11. Except as set forth in the preceding sentence, OncoPet Diagnostics, Inc. offers no guaranties of any type and does not assume any responsibility for the decisions made by the professional in charge of the patient or the animal care giver or the owner after receiving the results of an OncoPet RECAF™ test.
12. **Individuals, companies, not-for-profit organizations and any other entity or person requesting an OncoPet RECAF™ test acknowledge that the sole liability of the company and those involved in testing the samples is limited to the list price value of one test.**

# **APPENDIX A**

**ROC (Receiver Operating Characteristics) analysis.**

# ROC (Receiver Operating Characteristics) analysis.

If we plotted the normal values of RECAF or any other cancer markers (the example also works for blood sugar), we would find the bell-shaped curve shown below. Cancer patients, who have a higher blood RECAF value would also exhibit a bell-shaped curve but shifted towards higher values as shown in Figure 1.

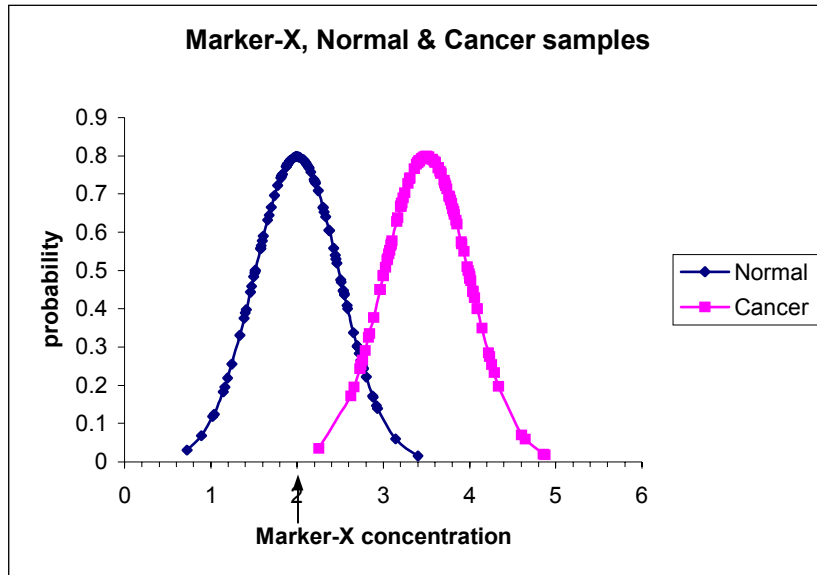


Figure 1 (points are fictitious)

The principle for a ROC analysis is simple: We take all the value (for both normal and cancer samples) and we look at the range they cover (0.4-5 in Figure 1). Then we divide that range into say 20 “thresholds” or properly called, cutoff values. For example, between 0.4 and 5.0 we could consider 20 cutoff values each 0.23 units higher than its predecessor. Next we choose the first cutoff value (in the example it would be  $0.4 + 0.23 = 0.63$ ) and we count the number of patients that we know have cancer and are above 0.63. Those are true positives (TP) for that cutoff value because they have cancer and they test positive. We also count the number of normal people below 0.63. Those are true negatives (TN) because we know they do not have cancer and they tested negative at that cutoff value. We might also have known cancer patients below 0.63 and those are false negatives (FN). Finally, we might have normals that test higher than 0.63 and those are false positives (FP). With those four parameters, we can then calculate the Sensitivity and the Specificity of the test for each cutoff value: The Sensitivity of the test is the number of cancers we catch (which is TP above) in all the cancer samples (detected or TP plus not detected or FN). Thus,  $\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$ . The Specificity of the test is the number of samples that test negative in the population of known normals and it can be expressed as  $\text{Specificity} = \text{TN} / (\text{TN} + \text{FP})$ .

Thus, we can easily calculate the Sensitivity and Specificity of the assay for each cutoff value. The data is usually represented in a table such as the one below:

Cutoff)	Sensitivity	Specificity	TP	TN	FP	FN
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<b>2.5</b>	100.0%	1.0%	162	1	102	0
<b>3.1</b>	100.0%	14.6%	162	15	88	0
<b>3.6</b>	100.0%	27.2%	162	28	75	0
<b>3.8</b>	100.0%	43.7%	162	45	58	0
...	...	...	...	...	...	...
...	...	...	...	...	...	...
<b>4.5</b>	97.5%	86.4%	158	89	14	4
<b>5.0</b>	95.7%	94.2%	155	97	6	7
<b>5.5</b>	88.3%	100.0%	143	103	0	19

Please note that as the cutoff value increases, so does the Specificity, at the expense of the Sensitivity, which moves in the opposite way.

It is now simple to select the best cutoff value above which we shall say a person tested positive: All we have to do is to find an acceptable Sensitivity and Specificity combination and use the corresponding value in the cutoff column.

It does not escape the attention of the reader that the larger the number of samples, the more accurate the results. Also, a large number of samples means that we can generate more thresholds with smaller intervals, which makes the choosing of the cutoff value more precise.

Since the tables are usually very large and numbers difficult to interpret at a glance, there is a way to plot these results called ROC (Receiver Operator Characteristics). In a ROC, for each cutoff value, the Sensitivity is plotted vs. the Specificity only that for visual purposes, the 100% (or 1) Specificity value is placed on the left and 0% is placed on the right. We then get a curve such as the one in fig 2.

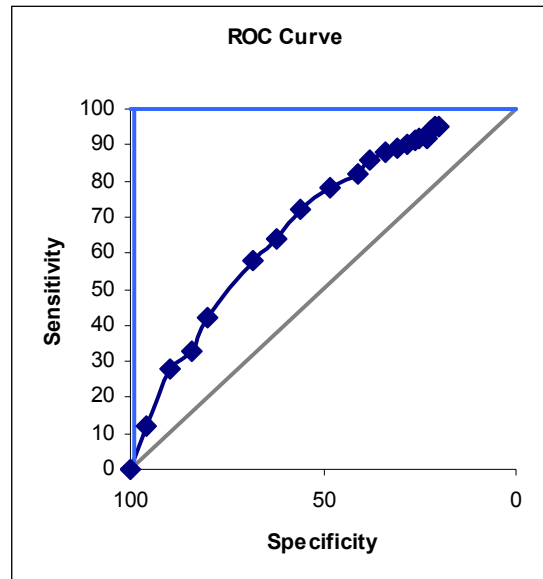


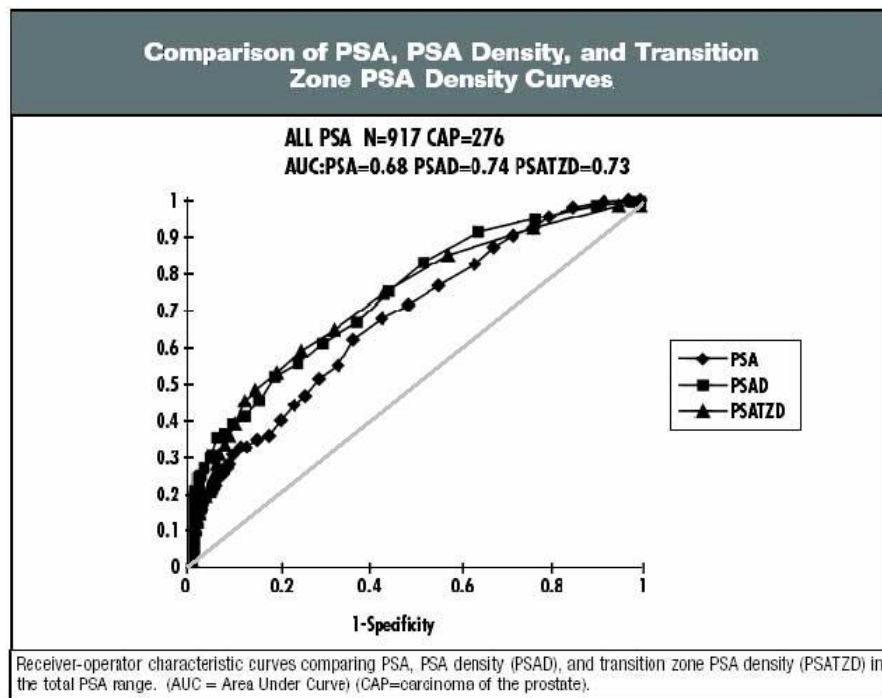
Figure 2

Please note that a complete lack of discrimination between cancer and non-cancer results in a diagonal line (represented in gray). On the other hand, if the assay discriminated

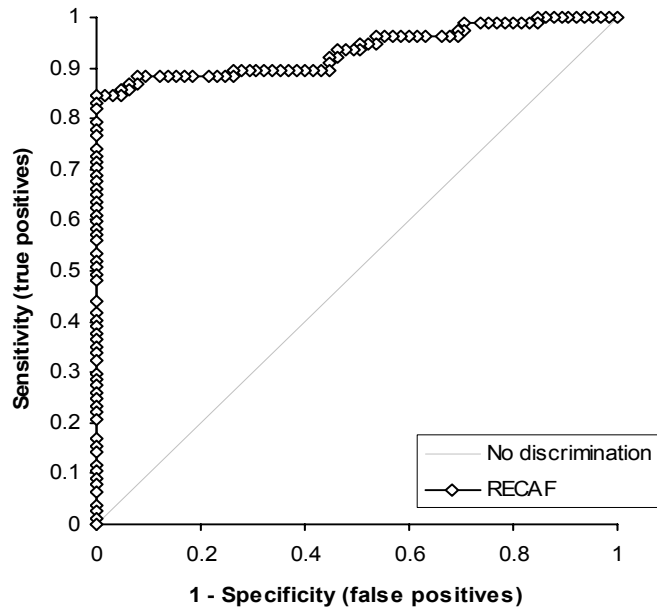
perfectly well (100% Sensitivity with 100% Specificity), then the curve would go up parallel to the vertical axis until it reached 100% (or 1) and then horizontally until the 100% value in the horizontal axis is reached. This is represented in light blue.

The actual curve splits the plot area in two and the area under the curve (AUC) can then be as low as 0.5 or as high as 1.0 of the total plotting area. Thus, the AUC is a measure of the quality of the test.

Now, by looking at the curve one can tell how good the discrimination is and by looking at the x and y values at the point of inflexion (if any), one gets a rough idea of the Specificity and Sensitivity of the test. Figure 3 shows an actual ROC curve for prostate cancer using PSA in its different flavors and Figure 4 the equivalent for prostate cancer obtained with RECAF. The AUC for PSA = 0.68 and for RECAF = 0.93.



**Figure 3**



**Figure 4**

# APPENDIX B

**Posters presented at different international meetings.**

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# RECAF: A New Broad-spectrum Cancer Marker With Diagnostic Potential

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**INTRODUCTION:** Alpha-fetoprotein (AFP), the first reported onco-fetal antigen of clinical value, is a major serum protein throughout foetal life [1], [2], [3], [4]. It is synthesized mainly by the fetal yolk sac and the liver [4], [5]. After birth, however, circulating AFP levels drop sharply, until it virtually disappears from blood in adult individuals [4]. Patients with hepatoma, ovarian and testicular teratocarcinomas, and with primitive gut tumors, on the other hand, exhibit high levels of AFP in serum, and this has been extensively used to develop diagnostic tests and for the delivery of anticancer drugs directly to the tumor cells [4], [6], [7], [2], [8].

Immature cells from most fetal tissues can internalize AFP, but this activity stops when the cells approach full differentiation, even when AFP levels in blood are still high and increasing [9], [10], [11]. Tissues from the three embryonic layers take up AFP [12], [10], [13]. In all cases, the uptake ceases when cells mature, regardless of the high concentration of AFP in the extracellular fluid [13], [15]. Tumor cells regain the ability to selectively internalize AFP *in vivo* and *in vitro*, suggesting the existence of a receptor-mediated mechanism which is present in undifferentiated cells of embryonic or tumor origin [7], [16], [17], [4].

Several groups attempted the isolation and characterization of AFP binding proteins/receptor molecules from cell cultures, tumor cells, and from freshly isolated human monocytes/macrophages [7], [14], [18]. Several membrane polypeptides (MW 18, 31, 50, 65 kD) have been proposed for the binding of AFP to the membranes of normal and/or cancer cells. These molecules bind liganded AFP (i.e. AFP bound to steroids, thyroid hormone, bilirubin, fatty acids, heavy metals, etc) and may be engaged in the transmembrane passage of such molecules into the endoplasmic reticulum (ER) and in their release into the cytoplasm [4], [19], [7], [11], [20], [14], [18]. To simplify the language, we have coined the term RECAF to reference any molecule that binds or acts as a receptor for AFP.

Since RECAF is present in cancer cells and in fetal cells, but not in their mature counterparts, it can be defined as an oncofetal antigen and therefore its detection should have clinical and research relevance [16], [7], [21], [11], [19]. This paper reviews the presence and distribution of RECAF in malignant tissues as opposed to normal or benign lesion specimens and describes the increased concentration of RECAF in the serum of cancer patients.

## MATERIALS AND METHODS:

**HISTOLOGY:** The BioCurex's Histo-RECAF™ kit - designed to stain 60 slides - was used following the instructions in its package insert. Tissue samples from patients and controls were fixed in 10% formalin and embedded in paraffin using routine procedures. Three 5 µm thick sections were cut from each sample and re-hydrated in PBS. The tissues were then incubated with 3% hydrogen peroxide in methanol for 15' to suppress endogenous peroxidase activity, washed and incubated at room temperature for 15' with the blocking agent provided with in the kit. The slides were then incubated for 45' with a dilution 1/10 of a Peroxidase-AFP conjugate. After washing, the color was developed with a mixture of DAB and H<sub>2</sub>O<sub>2</sub> (also included in the kit). After 5' the slides were rinsed with tap water, counterstained with Mayer's Haematoxylin, dehydrated, and mounted using Permount.

**SERUM RECAF ASSAY:** The soluble fractions of RECAF can be released from cancer cells either actively or after the cells die and therefore the circulating RECAF in cancer patients could be higher than in non-cancerous individuals. To test this hypothesis, we developed a competitive radio-immunoassay (RIA) using a polyclonal rabbit antibody reactive against the soluble fractions of RECAF. The rabbit antibody was generated by injecting 0.5-1 mg of RECAF purified from MCF-7 cells using an AFP-Sepharose chromatography column. The IgG fraction of the antiserum was purified on a Protein-A column and coated onto 96 well plates which were then blocked with 3% BSA. Soluble RECAF, purified from cancer cells in culture and labeled with <sup>125</sup>I, typical specific activities were 4-10 uCi/ug. 50 µl of a 100 ng/ml solution of <sup>125</sup>I-RECAF was mixed with 50 µl of the patient's serum and the mixture was incubated in the wells coated with the anti-RECAF antibody. After one hour, the wells were washed, detached from the plate's frame and the radioactivity was measured in a gamma counter. A calibrated cell extract containing RECAF was used throughout the studies as a standard and the determinations were expressed in RECAF Units.

## RESULTS:

**HISTOLOGY:** Figure 1 shows the RECAF staining in cancer samples and their normal or benign counterparts. Cancer cells appear positively stained and the staining is always cytoplasmic, with occasional capping of the cell membrane and of the nuclear membrane. The color intensity varies from one case to another, from one region to another within the same tumor or even within foci of cancer cells. Normal or benign tissues are almost completely negative with the occasional few individual cells presenting weak staining. In some cancer specimens, a few normal looking cells in the vicinity of malignant cells are stained weakly. In breast, both ductal and lobular carcinomas are positive. A slightly modified technique can be used to stain frozen sections (not shown) as well as fine needle biopsies. Fibroadenomas and other benign conditions (hyperplasia) were negative. In lung, adenocarcinomas, small cell carcinomas and squamous carcinomas were positive.

**SERUM RECAF:** Figure 2 shows the distribution of serum RECAF values for different cancers, benign tumors and 103 normal donors. The horizontal lines mark 2 cutoff values (95% and 99% specificity). Using those cutoff values, the sensitivity for each type of condition is summarized in Table I. Figure 3 depicts the ROC analysis of all the cancer sera combined vs. the benign and normal samples. The area under the curve = 0.988.

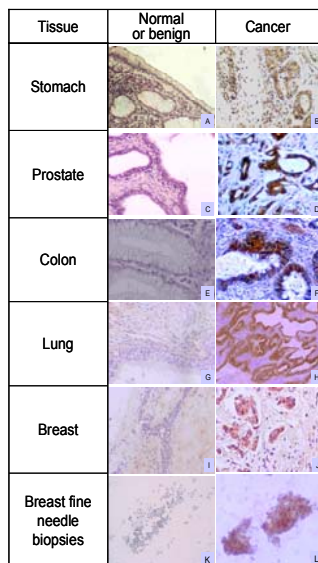


Figure 1

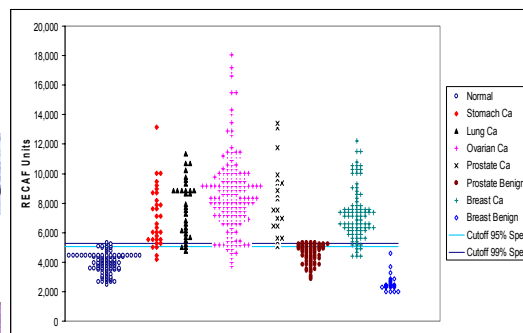


Figure 2

TABLE I

Cancer type	Sensitivity with 95% Specificity	Sensitivity with 99% Specificity	Number
Ovarian Ca	96%	92%	162
Stomach Ca	90%	87%	31
Lung Ca	91%	87%	32
Breast Ca	93%	90%	88
Prostate Ca vs. Normal	99%	95%	20
<b>TOTAL</b>	<b>94%</b>	<b>91%</b>	<b>333</b>
Breast Benign*	0	0	22
Prostate Benign*	25%	5%	77

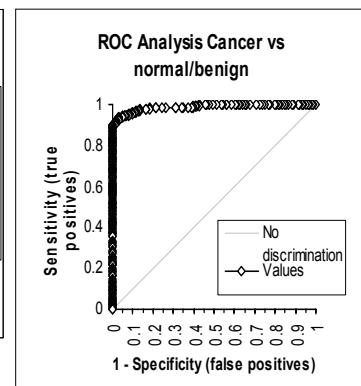


Figure 3

**CONCLUSIONS:** The results on tissue sections show a clear difference in the staining of cancer cells as opposed to normal or benign cells. This is of particular interest for automated systems using vision recognition where the computer time can be drastically reduced by examining only the brown "spots" (cells) on the slide.

In serum, RECAF values were elevated in all the types of cancer studied (breast, prostate, lung, stomach and ovary). Altogether, they represent 50% of all cancers in Occident. No cancer type has yet been found to be consistently negative and therefore it is likely that the RIA should also work in other malignancies. The assay detects approximately 90% of lung and breast cancers - the two prevalent types of malignancies. The vast majority of benign tumor samples were RECAF negative.

The fact that RECAF behaves as a rather sensitive and specific pan-cancer marker makes it suitable for routine screening at low cost.

The results shown herein indicate that RECAF has the potential to become a cancer marker of clinical significance. To assess the full extent of that potential, more samples from these and other types of cancer must be studied. We would like to invite colleagues who might be interested in developing collaborations to contact us.

1. Abelev GI, et al., Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation*, 1963; 1, p. 174-180.
2. Ruzsalski E and Szepplai M, Alpha-fetoprotein in cancer and fetal development. *Adv Cancer Res*, 1979; 29, p. 275-346.
3. Trojan J and Uziel J, Immunocytochemical localization of alpha-fetoprotein (AFP) and serum albumin (ALB) in ecto, meso, and endodermal tissue derivatives of the developing rat. *Oncodev. Biol. Med.*, 1983; 3, p. 13-22.
4. Deutsch HF, Chemistry and biology of alpha-fetoprotein. *Adv Cancer Res*, 1991; 56, p. 253-312.
5. Alpari E and Felzer E, Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. *Gastroenterology*, 1978; 74 (S P11), p. 856-858.
6. Ruzsalski E and Szepplai M, Studies of carcinofoetal proteins: III Development of a radioimmunoassay for AFP. *Demonstration of AFP in serum of healthy human adults*. *Int. J. Cancer*, 1971; 8, p. 374-378.
7. Uziel J, et al., Uptake of radiolabeled alpha-fetoprotein by mouse mammary carcinomas and its usefulness in tumor scintigraphy. *Cancer Res*, 1984; 44, p. 5314-5319.
8. Mizewski GJ, Alpha-fetoprotein structure and function: Relevance to isoforms, epitopes and conformational variants. *Exp. Biol. Med.*, 2001; 226, p. 377-408.
9. Moro R, Selective localization of AFP and serum albumin within the sensory ganglia cells of developing chicken. *Neuroscience Letters*, 1983; 41, p. 253-257.
10. Jacobson M, et al., Immunohistochemical evidence for intracellular localization of plasma proteins in CNS and some neural crest derivatives in human embryos. *Tumor Biology*, 1984; 5, p. 53-60.
11. Mizewski GJ, Alpha-fetoprotein binding proteins: Implications for transmembrane passage and subcellular localization. *Life Sci.*, 1995; 56, p. 1-9.
12. Moro R and Uziel J, Early localization of AFP in the developing nervous system of the chicken. *Oncodev. Biol. Med.*, 1981; 2, p. 391-398.
13. Moro R, et al., *In vivo* uptake of heterologous AFP and serum albumin in epigenital cells of developing chicken embryos. *Int. J. Dev. Neurosci.*, 1984; 2, p. 143-148.
14. Villacampa MJ, et al., AFP receptors in a human breast cancer cell line. *Bioch. Biophys. Res. Comm.*, 1984; 122, p. 1322-1327.
15. Molgarek K, et al., Immunohistochemical evidence for an intracellular localization of plasma proteins in human foetal choroid plexus and brain. *Neuroscience Letters*, 1979; 14, p. 85-90.
16. Uziel J, Redifferentiation and the fetal patterns of gene expression in cancer. *Adv Cancer Res*, 1983; 29, p. 127-174.
17. Savonere E, et al., Detection and measurement of alpha-fetoprotein in human breast cancer cytosol after treatment with 0.4 M potassium chloride. *Cancer Res*, 1983; 43, p. 3739-3741.
18. Suzuki Y, et al., Isolation and partial characterization of a specific alpha-fetoprotein receptor on human monocytes. *J Clin Invest*, 1991; 90, p. 1530-1536.
19. Navel J, et al., Cell-type specific receptors for alpha-fetoprotein in a mouse T-lymphoma cell line. *Proc. Natl. Acad. Sci. USA*, 1985; 82, p. 3301-3305.
20. Moro R, et al., Monoclonal antibodies directed against a widespread oncofetal antigen: The Alpha-fetoprotein Receptor. *Tumor Biology*, 1993; 14, p. 116-130.

# Breast Cancer

## Discrimination Of Malignant And Normal Serum Samples Using The Broad-Spectrum Cancer Marker RECAF

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**INTRODUCTION & MATERIAL AND METHODS:** Briefly, the receptor for AFP (RECAF) behaves like an oncofetal antigen present in all types of cancer so far studied but not significantly expressed by normal adult cells. Cancer cells release a soluble fraction of RECAF into the blood stream, where it can be detected with a RIA in which RECAF in the sample competes with <sup>125</sup>I-RECAF for binding to a rabbit antibody coated onto 96 well plates.

**SAMPLES:** Sera from 88 breast cancer patients, 22 patients with benign tumors, and 353 normal donors were tested with the Serum-RECAF™ assay. The benign and malignant nature of the lesions was confirmed histologically.

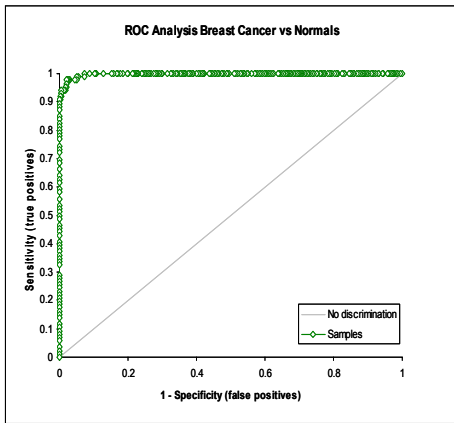
**RESULTS:** The results from an independent t-test comparing normal and cancer samples are shown in Table I. Figure 1 shows a ROC analysis of those two populations. The area under the curve is 0.997. Table II depicts the Sensitivity and Specificity of the ROC analysis for a cutoff value of 4,752 RECAF Units, the same value used in Part I for ovarian cancers. Figure 2 shows the distribution of the points, including the values of the 22 benign breast tumors. The horizontal line marks the cutoff value of 4,752 RECAF Units. Figure 3 shows that malignant cells stain positively for RECAF while benign tumor cells are negative.

**Table I**

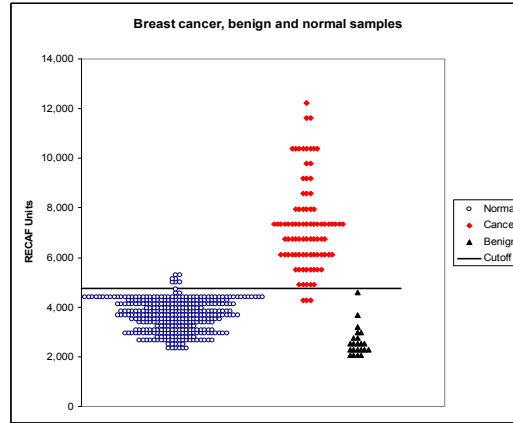
	Mean	S.D.
<b>Normals (353)</b>	<b>3,665</b>	<b>630</b>
<b>Cancers (88)</b>	<b>6,667</b>	<b>1,348</b>
	<b>t=25.66</b>	<b>d.f.=415</b>
		<b>p&lt;10<sup>-81</sup></b>

**Table II**

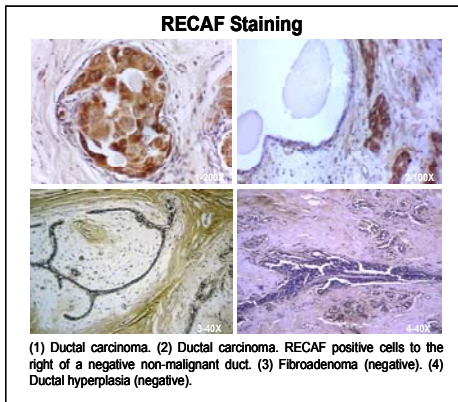
RECAF Units	Sensitivity	Specificity
<b>4,752</b>	<b>98.7%</b>	<b>97.5%</b>



**Figure 1**



**Figure 2**



**Figure 3**

**CONCLUSIONS:** Serum RECAF values are higher in patients with breast cancer than in normal donors. Samples from patients with benign tumors exhibit RECAF values similar to those of normal samples. This is consistent with the negative staining in benign breast lesions.

Both the sensitivity and specificity of the test are high. This suggests that a serum RECAF assay could be useful for detecting breast cancer as well as monitoring patients after treatment.

Using the same cutoff value, the results obtained on breast cancer samples are consistent with those obtained for ovarian cancer (see Part I).

# Discrimination Of Malignant And Normal Serum Samples Using The Broad-Spectrum Cancer Marker RECAF

**Lung  
Cancer**

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**INTRODUCTION & MATERIAL AND METHODS:** Briefly, the receptor for AFP (RECAF) behaves like an oncofetal antigen present in all types of cancer so far studied but not significantly expressed by normal adult cells. Cancer cells release a soluble fraction of RECAF into the blood stream, where it can be detected with a RIA in which RECAF in the sample competes with <sup>125</sup>I-RECAF for binding to a rabbit antibody coated onto 96 well plates. Paraffin tissue sections were stained with Peroxidase labeled AFP (Biocurex's Histo-RECAF™ kit).

**SAMPLES:** 22 serum samples from patients with lung cancer and 353 from normal donors were tested with the Serum-RECAF™ RIA. 24 tissue samples from lung cancers (8 adenocarcinomas, 8 small cell carcinomas and 8 squamous carcinomas, none related to the serum samples) were stained with BioCurex's Histo-RECAF™ kit.

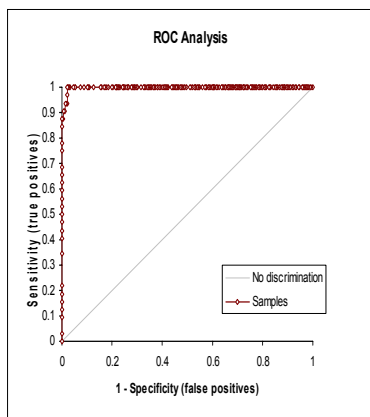
**RESULTS:** Table I shows the t-test results of comparing the RECAF serum values in samples from normal donors and cancer patients. Figure 1 shows a ROC analysis of those two populations. The area under the curve is 0.998. Table II depicts the Sensitivity and Specificity of the ROC analysis for a cutoff value of 4,752 RECAF Units, the same value used in Parts I & II for ovarian and breast cancers respectively. Figure 2 shows the distribution of the points and the horizontal line marks the cutoff value of 4,752 RECAF Units. Figure 3 shows the RECAF staining in three different types of lung cancer. Table III summarizes the staining intensity in 24 assorted lung cancers.

**Table I**

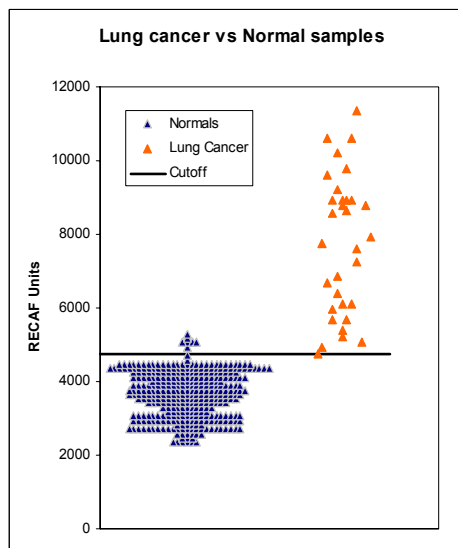
	Mean	S.D.
<b>Normals (353)</b>	<b>3,665</b>	<b>630</b>
<b>Cancers (22)</b>	<b>7,723</b>	<b>1,897</b>
	<b>t=23.96</b>	<b>d.f.=373 p&lt;3.2 x 10<sup>-73</sup></b>

**Table II**

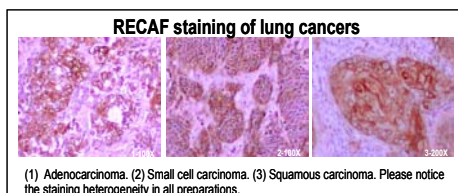
RECAF Units	Sensitivity	Specificity
<b>4,752</b>	<b>97%</b>	<b>97.5%</b>



**Figure 1**



**Figure 2**



**Figure 3**

**Table III**

	Strong positive	Positive	Negative
<b>Squamous carcinoma</b>	7/8	None	1/8
<b>Adenocarcinomas</b>	4/8	4/8	0/8
<b>Small cell carcinomas</b>	5/8	2/8	1/8

**CONCLUSIONS:** RECAF staining can be visualized in tissue sections of the three most common types of lung cancer. In serum, the concentration of RECAF is higher in lung cancer patients than in normal donors.

The sensitivity and specificity of the test are both above 90%, which suggests that the serum RECAF test could be used for screening and diagnosing lung cancer as well as for monitoring patients after treatment.

Using the same cutoff value, the results obtained in lung cancer are similar to those found in ovarian cancer (Part I) and breast cancer (Part II).

# Discrimination Of Malignant And Normal Serum Samples Using The Broad-Spectrum Cancer Marker RECAF

**Stomach  
Cancer**

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**INTRODUCTION & MATERIAL AND METHODS:** Briefly, the receptor for AFP (RECAF) behaves like an oncofetal antigen present in all types of cancer so far studied but not significantly expressed by normal adult cells. Cancer cells release a soluble fraction of RECAF into the blood stream, where it can be detected with a RIA in which RECAF in the sample competes with <sup>125</sup>I-RECAF for binding to a rabbit antibody coated onto 96 well plates.

**SAMPLES:** Sera from 31 stomach cancer patients (confirmed histologically) and 353 normal donors were tested with the Serum-RECAF™ assay.

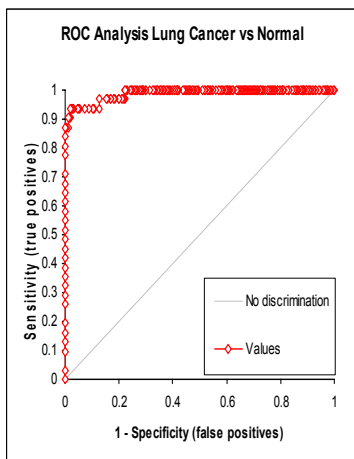
**RESULTS:** The results from an independent t-test comparing the RECAF concentration in the serum of normal and cancer patients are depicted in Table I. Figure 1 shows the ROC curve analysis of the data. The area under the curve is 0.988. Table II displays the Sensitivity and Specificity of the test using 4,752 RECAF Units as the cutoff value to discriminate between cancer and normal samples. Figure 2 shows the dispersion of the points. The horizontal line marks the cutoff value. Figure 3 shows the different RECAF staining of malignant cells compared to normal cells.

**Table I**

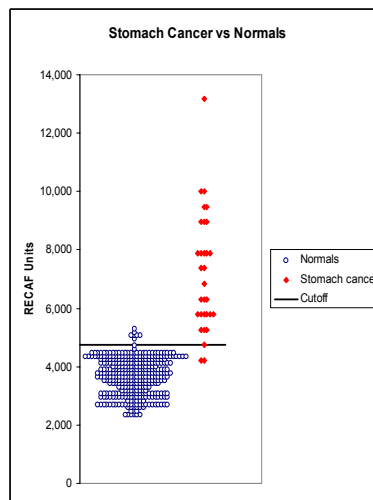
	Mean	S.D.
<b>Normals (353)</b>	<b>3,665</b>	<b>630</b>
<b>Cancers (31)</b>	<b>7,124</b>	<b>2,004</b>
	<b>t=20.09</b>	<b>d.f.=382</b>
		<b>p&lt;2.74 x 10<sup>-69</sup></b>

**Table II**

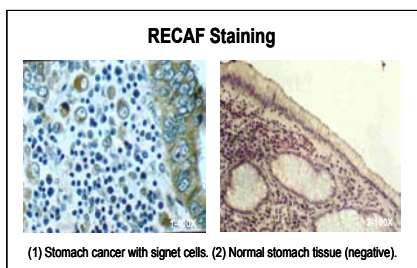
RECAF Units	Sensitivity	Specificity
<b>4,752</b>	<b>93.5%</b>	<b>97.5%</b>



**Figure 1**



**Figure 2**



**Figure 3**

**CONCLUSIONS:** Stomach cancer patients have significantly more circulating RECAF than normal donors. The assay exhibits high sensitivity and specificity and therefore it could prove useful for stomach cancer diagnosis and monitoring.

Moreover, using the same cutoff of 4,752 RECAF Units throughout this series, we have shown that approximately 90% ovarian, breast, lung and stomach cancers can be detected with a serum RECAF test. The aggregate number of cancer samples was 303 vs. 353 normal serum samples. The specificity was higher than 95% and the sera of 22/22 benign breast lesions were also negative. The positive staining of other types of cancers suggests that other types of malignancy should also be detectable by this serum test.

The consistency of the results obtained from one type of cancer to another in this series of studies indicate that circulating RECAF could be useful for routine cancer screening.